PATENT
Docket No. 416272002220
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:

Mycong-Jc CHO et al.

Serial No.: 09/552,252

Filing Date: April 18, 2000

For: COMPOSITIONS AND METHODS FOR PLANT TRANSFORMATION AND REGENERATION

Examiner: G. Helmer

Group Art Unit: 1638

DECLARATION OF MYEONG-JE CHO

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Mycong-Jc Cho, dcclarc as follows:

1. I am currently employed as a Vice President at Byotix, Inc. in Richmond, California and an Associate Specialist at the University of California at Berkeley.

2. I am a co-inventor of the invention disclosed in the above-referenced patent application, and am familiar with the contents thereof. I have assigned my rights in the invention to the Regents of the University of California ("the University") and stand to receive a small portion of any net royalties and fees received by the University in connection with the invention pursuant to my employment with the University.

3. I hold a Ph.D. in Agronomy from the University of Illinois at Urbana-Champaign, May 1991, and have been actively involved in Plant Physiology, Biochemistry and Molecular Biology research for over 20 years. My curriculum vitae is attached hereto as Exhibit A.

4. I am of the opinion that the term "green regenerative tissue" (also termed highly regenerative, green tissue in our several publications) as it is used in this application is clearly defined. Green regenerative tissue is a term that we came up with to describe a particular tissue type that is morphologically distinct from callus tissue as shown in Panels A and E of the attached figure. Applying the plant culturing techniques in the patent application will yield green regenerative tissue. Culturing a plant tissue like callus under the right conditions will produce sectors of green, shiny, nodular and compact tissue. This tissue is different from the surrounding tissue as is described in the patent application. One of skill in the art can grow plant tissue under the conditions in the patent application to produce the green regenerative tissue. Once produced, they would be able to readily differentiate the surrounding callus tissue from green regenerative tissue by observing the morphology (appearance) of the tissue. The tissue selected by its morphology can then readily be proliferated and regenerated into whole plants.

5. I have used the techniques described in the patent application to generate examples of normal callus and green regenerative tissue. Photographs of the tissues have been included with this declaration. Panel A shows two-month-old normal embryogenic tissue of Galena (i.e., callus tissue produced by a standard method that is similar to the protocol described by Wan and Lemaux in Plant Physiology (1994)). Panel B shows four-month-old green, highly regenerative tissue of Galena (i.e., green regenerative tissue produced by the modified culture media plus dim light methods of the present claimed invention). As the name suggests, the tissue in Panel B is a distinctly different color and appearance from the tissue in Panel A (see also Panel E). In addition, the tissue in Panel B has a different morphology. The tissue in panel B is green, shiny and nodular just as described in the patent application.

6. The green regenerative tissue of Galena was initiated DBC2 medium (Panel E) and maintained on DBC3 medium (Panel B). As described in the patent application, DBC2 medium is a culture medium containing 2.5 mg/L 2,4-D (an auxin), 0.1 mg/L 6-benzylaminopurine (BAP, a cytokinin), 5.0 μM (50X) copper, and maltose as a carbon source; and DBC3 medium contains 1.0 mg/L 2,4 D, 0.5 mg/L BAP, 5.0 μM copper and maltose. The plant tissue was exposed to dim light of 10-30 $\mu E\ m^{-2}\ s^{-1}$ for four months to initiate and proliferate the green regenerative tissue. The callus tissue in Panel A was cultured on a standard callus induction medium containing 2.5 mg/L 2,4-D, 0 mg/L BAP, 0.1 μM (1X) copper, and maltose as a carbon source. The plant tissue was incubated in the dark for two months to

generate the callus tissue as described by Wan and Lemaux (1994). The longer the incubation period, the worse the morphology of the tissue.

7. Results of culturing and regeneration of the two tissues is shown in Panel C and D. The same medium (FHG) and light conditions (~50 $\mu E\ m^{-2}\ s^{-1}$) were used for regeneration of both tissues. Panel C shows plant regeneration from the callus tissue in Panel A. Panel D shows plant regeneration from the green regenerative tissue in Panel B. These panels show that the green regenerative tissue is highly regenerative, especially when compared to the callus tissue produced by standard methods. Panel C shows no regeneration of green plants while Panel D shows a significant degree of regenerated green plants sprouting from the green regenerative tissue. Galena is a commercial cultivar for malting, but is very recalcitrant in tissue culturing using standard methods. However, the results in Panel D are typical of the results obtained with Galena using the methods of the present invention.

8. The green regenerative tissues generated by the protocol described in the patent application could be used directly as transformation targets. In addition, when this green regenerative tissue protocol was used as an intermediate step between callus induction and regeneration, remarkable improvement in regenerability was observed as described in the patent application.

9. I have read and understand the subject matter disclosed in Wan, Y. and Lemaux, P.G., Plant Physiology (1994) 104:37-48 ("Wan and Lemaux").

10. Wan and Lemaux disclose on page 38, under "Plant Materials", growing barley plants of a winter cultivar, Igri, of 10 cm in height under light of 10-15 μE at 4° C for 8 weeks. As I understand this section, it refers to cultivating whole plants and not plant tissues or seeds. The cultivation is intended to vernalize the plants. Vernalization is the process of exposing a plant in the vegetative state of growth to lowered temperatures and light conditions to induce or accelerate flowering in the plant. The low temperature (4° C) is certainly not optimal for tissue culture. A room temperature range of between 24° C and 27° C is optimal for tissue culture for most plant species including barley. Thus, the vernalization conditions for whole plants described in Wan and Lemaux would not be useful for tissue culture, nor would they provide any insight into optimal tissue culture conditions.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

January 21, 2004 Myeong Je Cho

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EDUCATION/TRAINING/EXPERIENCE

Seoul National University S. Korea	B.S., *cum laude*	1977-1984	Agronomy
Korean Army S. Korea		1981-1983	
Seoul National University S. Korea	M.S.	1984-1986	Agronomy
University of Illinois Urbana, IL	Ph.D.	1987-1991	Agronomy (Soybean Physiology and Biochemistry)
University of Illinois Urbana, IL	Postdoctor	1991-1994	Soybean Molecular Biology
University of California Berkeley, CA	Postdoctor	1994-1997	Cereal Transformation & Gene Expression
University of California Berkeley, CA	Assistant Researcher	1998- 1999	Cereal Transformation & Gene Expression
Ventria Bioscience Sacramento, CA	Consultant	May 1999- Nov. 1999	Research Consultation
Scigen Harvest Seoul, S. Korea	Consultant	Dec. 1999- Nov. 2000	Research Consultation
Exelixis, Inc. San Francisco, CA	Consultant	Oct. 2000- Sep. 2001	Research Consultation
Rural Development Administration S. Korea	Honorary Scientist	Oct. 1998-	Research Consultation
Genomine, Inc. S. Korea	Scientific Advisor	Jun. 2002-	
University of California Berkeley, CA	Associate Specialist	1999-	Crop Transformation & Gene Expression
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PUBLICATION

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PATENT APPLICATIONS

Lemaux PG, Buchanan BB, **Cho M-J**, Kim H-K. Cloning and characterization of glucose-6-phosphate dehydrogenase gene from barley.***

Wong J, **Cho M-J**, Lemaux PG, Buchanan BB. Change in redox status and abundance of alpha-amylase inhibitor proteins in transgenic wheat overexpressing thioredoxin *h*.***

Cho M-J, Wong J, Lemaux PG, Buchanan BB. Transgenic wheat grains overexpressing wheat thioredoxin *h*.***

Wong J, **Cho M-J**, Buchanan BB, Lemaux PG. Value-added traits in barley grain transformed with thioredoxin *h*.***

Cho M-J, Wong J, Marx C, Buchanan BB, Lemaux PG. Enhanced expression of thioredoxin in plant seeds.***

Lemaux PG, **Cho M-J**. Novel methods for the transformation of multiple genotypes of wheat, oat, maize, rice and turf/forage grasses.*

Cho M-J, Lemaux PG, Buchanan BB. Production of heterologous proteins in maturing monocot seeds (US patent issued)

Zhang S, **Cho M-J**, Bregitzer P, Lemaux PG. Methods and compositions for transformation of cereals using cultured meristematic tissue (US patent issued)

Cho M-J, Lemaux PG, Buchanan BB. Cloning and characterization of NADP-thioredoxin reductase gene from barley (US patent issued)**.

Calliu M, Del Val G, **Cho M-J**, Lemaux PG, Buchanan BB. Barley gene for thioredoxin (US patent issued)**.

Lemaux PG, **Cho M-J**. Novel methods for the transformation of multiple genotypes of barley (US patent issued)*.

* combined for PCT filing.
** combined for US patent and PCT filing.
*** combined for PCT filing.

MANUSCRIPTS IN PREPARATION

Cho M-J, Jung HR, Calliu M, Del Val G, Lemaux PG, Buchanan BB. Isolation and characterization of barley genes for thioredoxin *h* and NADP-thioredoxin reductase.

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Choi HW, Lemaux PG, **Cho M-J**. Chromosomal variation in transgenic plants.

Cho M-J, Lemaux PG. Rapid isolation of a cDNA clone by PCR.

ONGOING RESEARCH PROJECTS

Cho M-J, Okamoto D, Kim Y-B, Yoo H-S, Lemaux PG, Buchanan BB. Improvement of baking quality by application of thioredoxin system.

Cho M-J, Yano H, Lemaux PG, Buchanan BB. Improvement of grain quality in transgenic rice

plants.

Yoo HS, Lemaux PG, **Cho M-J**. Application of *Ac/Ds* system for excision of selectable marker gene in transgenic wheat.

Cho M-J, Buchanan BB, Lemaux PG. Strategy for enhancing expression of thioredoxin *h* in transgenic barley grains.

Yu X-H, Bregitzer P, **Cho M-J**, Chung ML, Lemaux PG. Transposon-mediated generation of marker-free barley plants expressing putative antifungal proteins.

ABSTRACTS

Yu X-H, Bregitzer P, **Cho M-J**, Chung ML, Yu HS, Lemaux PG (2003) *Ac/Ds* Transposon-mediated generation of marker-free barley plants expressing scab resistance proteins. 2003 ASPB Meeting. Honolulu, Hawaii

Cho M-J, Jung H-R, Kim H-K, Caillau M, del Val G, Lemaux PG, Buchanan BB (2003) Gene isolation and characterization of thioredoxin *h* and NADP-thioredoxin reductase from barley (*Hordeum vulgare* L.). 2003 ASPB Meeting. Honolulu, Hawaii

Kim H-K, **Cho M-J**, Jung HR, Y-B Kim, Morigasaki S, Wong JH, Lemaux PG, Buchanan BB (2003) Alleviation of wheat allergenicity using thioredoxin system. 2003 Congress on In Vitro Biology, Portland, OR

Godwin I, Byrne E, Zhang S, Cho M-J, Lemaux PG (2003) Retrotransposons and cereal genome instability: BARE-1 activation in the genetic transformation of barley. XIX International Congress of Genetics, Melbourne, Australia, July 6-11, 2003.

Kim H-K, **Cho M-J**, Jung HR, Y-B Kim, Morigasaki S, Wong JH, Lemaux PG, Buchanan BB (2002) Lowering the allergenicity of wheat. 2002 Wheat Industry Forum, Albuquerque, NM

Yu X-H, Bregitzer P, **Cho M-J**, Chung ML, Lemaux PG (2002) Transposon-mediated generation of marker-free barley plants expressing putative antifungal proteins. 2002 National Fusarium Head Blight Forum Proceedings. Erlanger, KY, Dec. 7-9, 2002

Cho M-J, Buchanan BB, Lemaux PG (2001) Use of barley endosperm-specific hordein promoters for production of recombinant proteins in transgenic cereal seeds. 2001 Congress on In Vitro Biology

Cho M-J, Lemaux PG (2001) An efficient system for transformation and plant regeneration of sorghum (*Sorghum bicolor* L.) using highly regenerative, green tissues. 2001 Congress on In Vitro Biology

Cho M-J, Yano H, Okamoto D, Le VK, Newcomb KL, Buchanan BB, Lemaux PG (2001) High-frequency transformation of rice (*Oryza sativa* L.) via microprojectile bombardment of mature seed-derived highly regenerative tissues. 2001 Congress on In Vitro Biology

Choi HW, Lemaux PG, **Cho M-J** (2001) Long-term stability of transgene expression driven by barley endosperm-specific hordein promoters in transgenic barley (*Hordeum vulgare* L.) plants. 2001 Congress on In Vitro Biology

Choi HW, Lemaux PG, **Cho M-J** (2001) Transformation process exacerbates cytological variation in transgenic grass and cereal plants. 2001 Congress on In Vitro Biology

Yu X-H, Bregitzer P, **Cho M-J**, Lemaux PG (2001) Ac-Ds System to obtain marker-free transgenic barley plants that stably express putative antifungal proteins. 2001 National Fusarium Head Blight Forum Proceedings. Erlanger, KY, Dec. 8-10, 2001

Wong JH, Ren P-H, Cai N, Kim H-K, **Cho M-J**, Lemaux PG, Buchanan BB (2001) Thioredoxin-linked proteins in transgenic cereals over-expressing thioredoxin. 2001 Annual Meeting of the American Society of Plant Physiologists

Cho M-J (2000) Development of transformation systems for monocotyledonous crop species using highly regenerative tissues. 2000 Congress on In Vitro Biology

Cho M-J, Kim H-K, Choi HW, Buchanan BB, Lemaux PG (2000) Endosperm-specific expression driven by barley D-hordein promoter and its inheritance in transgenic barley and wheat plants. 2000 Congress on In Vitro Biology

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Wong JH, Ren P-H, Cai N, **Cho M-J**, Buchanan BB (2000) Transgenic barley grain over-expressing wheat thioredoxin *h* shows improved germination properties. 2000 Annual Meeting of the American Society of Plant Physiologists

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Choi HW, Lemaux PG, **Cho M-J** (1999) High frequency of cytogenetic aberration in transgenic oat (*Avena sativa* L.) plants. 1999 Congress on In Vitro Biology

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Maughan PJ, Philip R, **Cho M-J**, Widhom JM, Vodkin LO (1998) Biolistic transformation, expression and inheritance of bovine ß-casein in soybean. 1998 Congress on In Vitro Biology

Cho M-J, Jiang W, Lemaux PG (1998) Transformation of recalcitrant barley cultivars: improvement of regenerability and decreased albinism. 1998 Congress on In Vitro Biology

Cho M-J, Ha CD, Buchanan BB, Lemaux PG (1998) Subcelluar targeting of barley hordein promoter-*uidA* fusions in transgenic barley seed. 1998 Congress on In Vitro Biology

Cho M-J, Zhang S, Lemaux PG (1998) Transformation of shoot meristem tissues of oat using three different selectable markers. 1998 Congress on In Vitro Biology

Cho M-J, Jiang W, Lemaux PG (1998) High-frequency transformation of oat via microprojectile bombardment of embryogenic callus-derived highly regenerative cultures. 1998 Congress on In Vitro Biology

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RESEARCH GRANTS

Development of a Gene Expression System in Soybean. PI, Myeong-Je Cho
(November 2002-October 2005) $700,000

Genetic Engineering of Waterlogging-resistant Barley Plants. PI, Peggy G. Lemaux & Myeong-Je Cho
(Submitted)

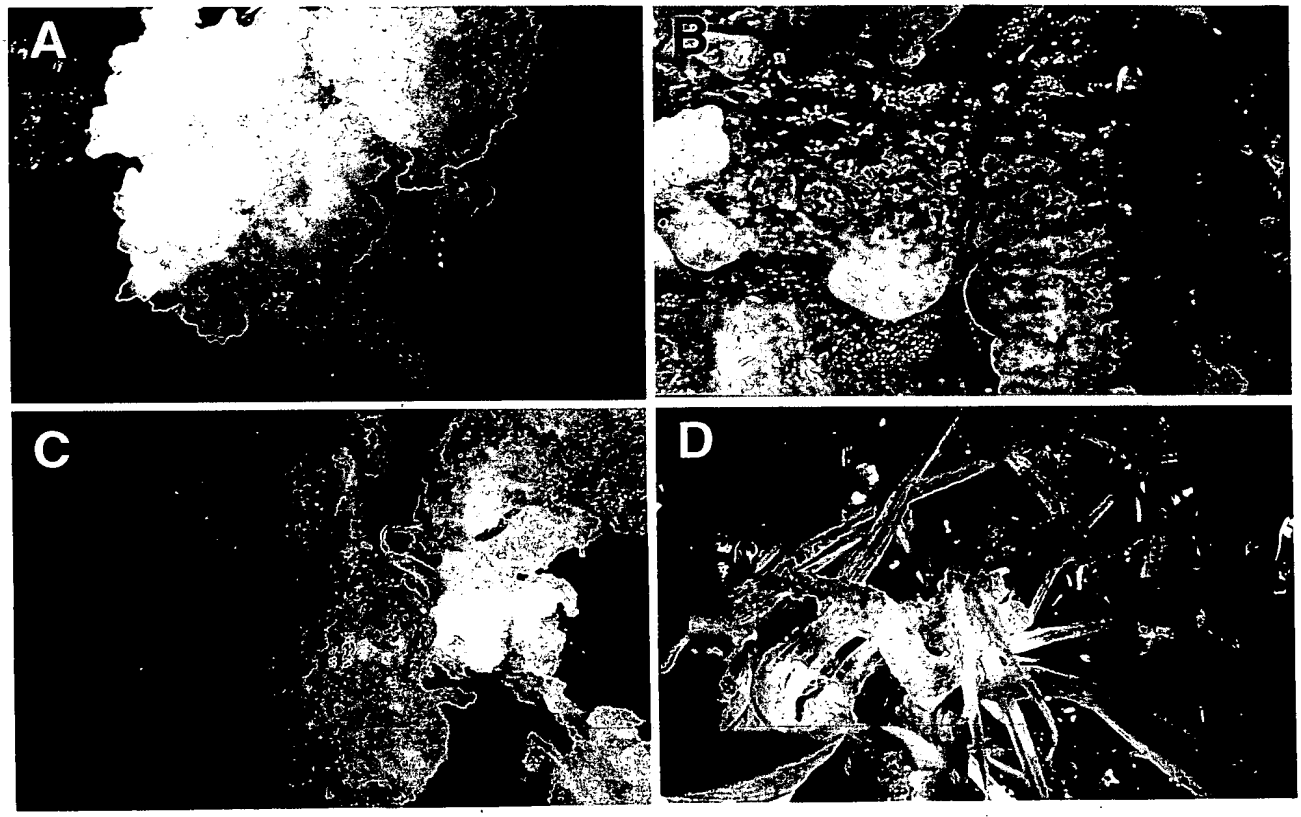
ACADEMIC HONORS

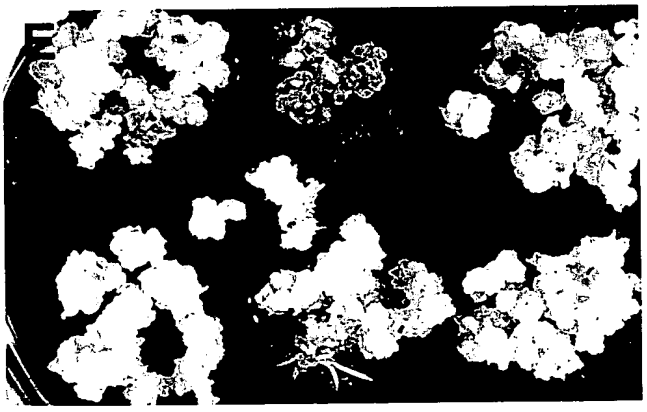
Graduation *cum laude* (February 1984)
Korean Government Scholarship (December 1986 - December 1989)

MEMBERSHIPS IN PROFESSIONAL ORGANIZATIONS

American Society of Plant Physiologists

Figure for Myeong-Je Declaration





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